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Host-parasitoid relationships within figs of an invasive fig tree: a fig wasp community structured by gall size

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Abstract. 1. Revealing the host specificity of the predators/parasitoids of invasive species is a prerequisite when assessing the suitability of biocontrol agents, while the host ranges of top predators are likely to vary among communities comprising different species.

2. *Ficus microcarpa* is a native plant in Asia and Australasia and has invaded in sometropical and subtropical areas. Besides its species-specific pollinator, its figs also support many ovule-galling and parasitoid non-pollinating fig wasps.

3. Here, based on a global collection of fig wasps associated with *F. microcarpa* figs, we used path analysis, supplemented by within-fig spatial distributions and natal gall sizes to reveal food web structure of its associated fig wasps and the factors forming host ranges of parasitoids.

4. The fig wasp community was species-rich, and parasitoids were far rarer in the plant's introduced range. Parasitoids exhibited some host specificity, and four specific natural enemies of the plant's pollinator were identified with various intensities of effects on pollinator abundance. Parasitoid host ranges were consistent in both ranges of the plant, and mainly restricted by the size and the locations of host galls. No parasitoids were found associated with a unique seed predator.

5. Our results showed how a large number of fig wasp species partition one host fig tree's figs and identified the species that have potential to control the sexual reproduction of *F. microcarpa*.

Key words. Biocontrol, *Ficus*, fig wasps, food web, gall size, host range, parasitoid.

Introduction

Modification and simplification of food webs by human activities can cause the collapse of local communities (Tylianakis *et al*., 2007; Estes *et al*., 2011) and can facilitate biological invasions (Dickie *et al*., 2010; Gurevitch *et al*., 2011). Utilising host-specific predators/parasitoids from the native ranges of invasive species is the basis for classical biological control projects, and an understanding of their host ranges and relationships with other species based on the food web structure is a prerequisite when assessing the suitability of agents for deliberate introduction (Keane & Crawley, 2002). Furthermore, host ranges

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of predators/parasitoids are likely to vary in communities with different species compositions (Keane & Crawley, 2002), and thus it is necessary to evaluate the consistency of host specificity of candidate biological agents and gain an understanding of the factors restricting their host ranges.

The species-rich genus *Ficus* is a significant contributor to the sustainability and biodiversity of tropical and subtropical forest ecosystems (Herre *et al.*, 2008; Compton *et al.*, 2010), but a small number of *Ficus* species have also been introduced outside their natural ranges and become invasive (Richardson *et al.*, 2000; Caughlin *et al.*, 2012). The wider significance of fig trees results from the large number of vertebrates that feed on their figs and disperse their seeds (Shanahan *et al.*, 2001). Figs are complex hollow inflorescences containing tiny male and female flowers on their inner surfaces. Sexual reproduction of the plants relies on adult female pollinating fig wasps (Agaonidae), whose offspring develop in galled ovules in figs (Cook & Rasplus, 2003; Liu *et al.*, 2015). Pollinators are almost always host-tree-specific and one or a small number of fig wasp species typically pollinate each tree (Chen *et al.*, 2012; Cruaud *et al.*, 2012).

Besides pollinating agaonids, figs are also exploited by large numbers of non-pollinating fig wasps (NPFW) belonging to Agaonidae and other families of Chalcidoidea (Eurytomidae, Ormyridae, Pteromalidae and Torymidae) (Cook & Rasplus, 2003; Cook & Segar, 2010; Wang *et al.*, 2015a). Like pollinating fig wasps, most NPFW are believed to have one or only a few host fig tree species (Cook & Segar, 2010; Li *et al.*, 2010; Zhou *et al.*, 2012). Females of most NPFW lay their eggs from outside the figs, and their offspring develop in galled ovules and emerge from the figs at the same time as those of the pollinators. NPFW can be allocated into two trophic levels comprising phytophages with larvae that only feed on plant tissues and do not directly kill other fig wasps and parasitoids with larvae that develop at the expense of other species (Cook & Segar, 2010; Segar & Cook, 2012). Most species in Pteromalidae subfamilies Epichrysomallinae and Otitesellinae are believed to be gall formers, and Eurytomidae and Sycoryctinae (Pteromalidae) species are generally regarded as parasitoids of epichrysomallines and agaonids, respectively (Compton, 1993b; Segar & Cook, 2012; Suleman *et al.*, 2013), but exceptions to broad taxonomic generalisations are likely (Pereira *et al.*, 2007; Compton *et al.*, 2009; Chen *et al.*, 2013; Wang *et al.*, 2014; Krishnan *et al.*, 2015). Nevertheless, the exact extent of parasitoid host specificity within each fig wasp community associated with a particular fig tree is poorly understood.

Related fig trees are often pollinated by related agaonids, suggesting that they share long co-evolutionary histories (Cruaud *et al.*, 2012). Some gall-forming NPFW show similar patterns, but parasitoids often appear to be more likely to display host or niche shifts (Cook & Segar, 2010; Segar *et al.*, 2013). Nonetheless, niche conservatism induced by morphological characters such as fig size, ovipositor length (reflected by locations of galls inside figs) and gall size contributes to the matches between parasitoids and their hosts, indicating the role of evolutionary constraints in the structuring of fig wasp communities (Dunn *et al.*, 2008; Segar & Cook, 2012; Segar *et al.*, 2013).

Ficus microcarpa is a monoecious fig tree that has been widely planted outside its native range. Where the plant's pollinator is also introduced, it has increasingly become invasive (Wang *et al.*, 2015b). Numerous NPFW species can exploit its figs and some can significantly reduce the plant's seed production (Wang *et al.*, 2014, 2015a,b), but their value as potential biocontrol agents depends on an understanding of their trophic relationships. To address this, we sampled the fig wasps associated with the figs of *F. microcarpa* throughout much of the plant's native and introduced ranges and recorded the sizes and spatial locations of their natal galls within individual figs with the aims of (i) detecting and comparing host ranges of parasitoid fig wasps between the two ranges of *F. microcarpa* and (ii) testing the factors that contribute to fig wasp community structure. Specifically, we asked (1) whether parasitoids are restricted to particular hosts, (iii) whether parasitoid host ranges varied between different ranges of the plant, (iv) whether the size of galled ovules determines which parasitoids utilise them and generates partially or complete compartments within the food webs and (v) whether the fig wasps that develop in seeds, rather than galls, support a distinct suite of parasitoids.

Materials and methods

Study system

Ficus microcarpa is a monoecious fig tree with a natural distribution in tropical and subtropical forests of SE Asia and Australasia, where it grows as a strangler or from bare rocks (Berg & Corner, 2005). During the last 200 years, it has also been transplanted widely as an ornamental and shade tree into many tropical and warm temperate urban areas (Wang *et al.*, 2015a,c). A crop of *F. microcarpa* can consist of up to several thousand small figs located in the leaf axils, and mature figs are eaten by a wide range of bird species that aid rapid expansion of *F. microcarpa* populations (Shanahan *et al.*, 2001; Caughlin *et al.*, 2012). It has been regarded as invasive in Bermuda, Florida and Hawaii and is an expanding nuisance species in urban environments elsewhere (Wang *et al.*, 2015b).

As with other fig trees, sexual reproduction of *F. microcarpa* is recorded as depending on a host-specific pollinating fig wasp, namely *Eupristina verticillata* Waterston. However, within the native range this taxon represents a complex of several cryptic pollinating species and also one species (*Eupristina* sp. 'cheater') that no longer pollinates the plant (Sun *et al.*, 2011; Wang *et al.*, 2014). Only one of these is known to have been introduced outside the native range (R. Wang, unpublished).

Figs of *F. microcarpa* are exploited by a large community of NPFW comprising at least 42 species (Wang *et al.*, 2015a). Except for the non-pollinating agaonid, all the known NPFW belong to families of Chalcidoidea other than Agaonidae and lay their eggs in the ovules or seeds via the outer wall of the fig by utilising their long ovipositors (Cook & Segar, 2010). Like the agaonids, a single NPFW larva typically completes development inside each ovule. *Philotrypesis taiwanensis* (Sycoryctinae) is an exception as it is an obligate seed predator, with larvae that consume seeds rather than hosts in galled ovules (Wang *et al.*, 2014). The NPFW are generally specific to *F. microcarpa,* but a few species may be associated mainly with closely related *Ficus* species and only occasionally utilise this host (Zhou *et al.*, 2012; Wang *et al.*, 2015a).

Sample sites and fig wasp faunas

Fig crops were sampled in both the introduced and native ranges of *F. microcarpa*, with seven native range sites located in East and Southeast Asia and 20 sites in the plant's introduced range (Tables S1, Fig. 1a). From December 2010 to July 2013, several *F. microcarpa* crops were sampled at each site, with 10–30 mature figs (depending on crop size) being haphazardly selected from all heights of each target tree, and all sampled figs were stored in 70% ethanol. When dissecting figs, all flowers were identified under a binocular microscope, and were sorted into the following categories: male flowers, seeds, unfertilised and undeveloped female flowers, galls containing wasps, and failed, empty galls. All fig wasps were identified morphologically using primarily Chen *et al.* (1999) and Feng and Huang (2010), or scored as morphospecies where necessary (Wang *et al.*, 2015a). The higher taxonomy of fig wasps was based on the information shown in figweb (http://www.figweb.org).

Gall sizes

We randomly selected 105 figs (from 22 crops) collected from Panzhihua, Xichang, Xishuangbanna, Taibei and Manila. At least five galls with adult fig wasp offspring were sub-sampled in each fig and their lengths and widths were measured to the nearest 0.04 mm under a dissecting microscope using an eyepiece graticule. Fig wasps inside the measured galls were then identified. The volumes of the galls were calculated assuming their shape to be an ellipsoid.

Spatial stratification of fig wasps

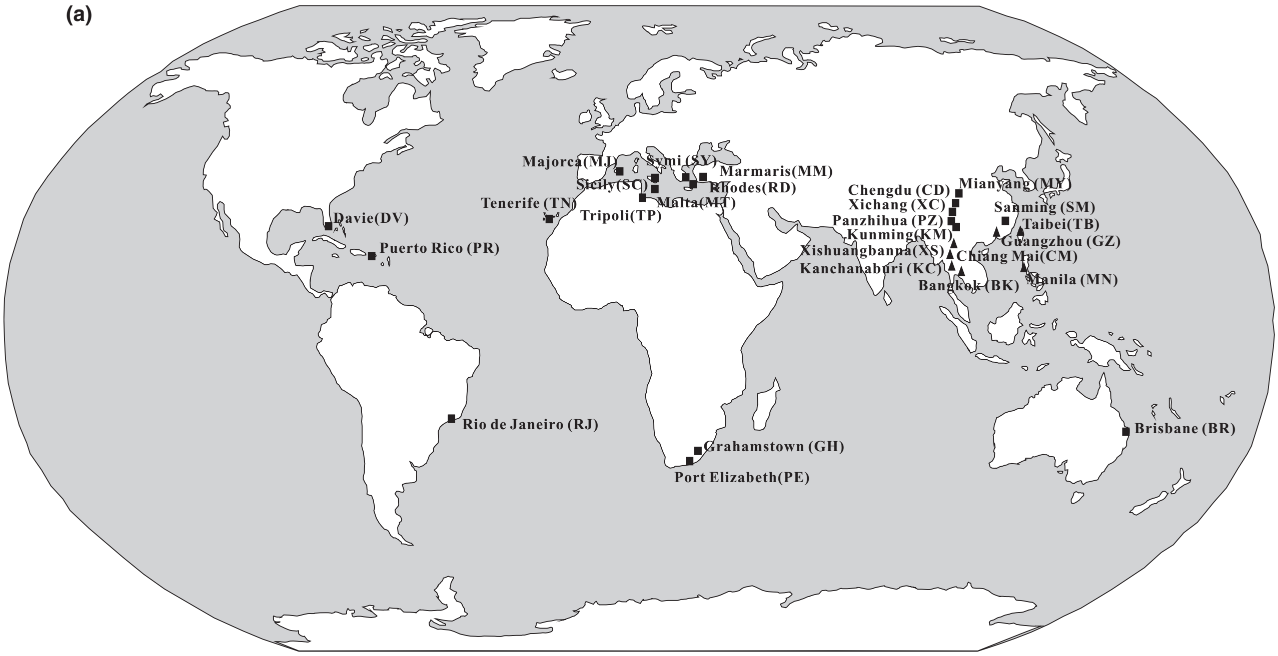
Pedicels elongate after their associated ovules are galled and their lengths can be used to delineate the spatial distribution of the galls in mature figs. Ovules with longer pedicels are located closer to the centre of a fig (Dunn *et al.*, 2008; Yu & Compton, 2012). We recorded pedicel lengths in 33 figs from seven *F. microcarpa* crops collected in Xichang, Xishuangbanna, Bangkok and Kanchanaburi. Pedicel lengths and the contents of their associated ovules were recorded from all the flowers that either developed seeds or were galled. Each fig contained at least three galls occupied by putative parasitoids. Pedicel lengths were measured to the nearest 0.02 mm under a dissecting microscope using an eyepiece graticule and the adult fig wasps inside the galls were then identified.

Statistics

Path analysis. We assigned the fig wasps associated with *F. microcarpa* into two trophic levels, putative phytophages with larvae that feed exclusively on plant tissue: (mainly ovule gallers but including the obligate seed-feeder, *P. taiwanensis*) and putative parasitoids with larvae that develop at the expense of gall-forming species (Wang *et al.*, 2015a). The hypothesised relationships between different fig wasp species and seeds in the path analysis model were set as follows (Fig. 1b):

1. Putative parasitoids were selected initially on the basis of their long ovipositors, supported where possible by experimental data (Rodriguez *et al.*, 2015). This indicates that they lay their eggs into older, larger figs that had been pollinated some time before (Compton *et al.*, 1994; Segar *et al.*, 2013). Parasitoids were expected to negatively influence their host fig wasps (one or more phytophagous species) in the path analysis without affecting seed numbers (Kerdelhué *et al.*, 2000). If any putative parasitoids were found to reduce seed production in the path analysis, then this would suggest they were atypical late-ovipositing phytophages rather than parasitoids. Their negative effects on seed production could then be indicative of either their galls competing with seeds for nutrients, of seed-feeding species that utilise pollinated ovules, or of species with a mixed feeding strategy that combines utilisation of both gall-forming fig wasps and seeds as hosts (Pereira *et al.*, 2007).
2. Depending on the relative timing of their oviposition, early-ovipositing ovule gallers could potentially have negative effects on other phytophages because they are competing for ovules to utilise and later through competition for nutrients (Wang *et al.*, 2015b). The pollinator clearly facilitates the seed predator. Pollinators were especially likely to be adversely affected by the 'cheater' *Eupristina* sp. in shared figs and vice versa, because individual females of these species concentrate their oviposition within a single fig after the females enter to oviposit.
3. All non-pollinating phytophages have the potential for negative impacts on seed production via both competition for oviposition sites and later for competition

(a)



(b)

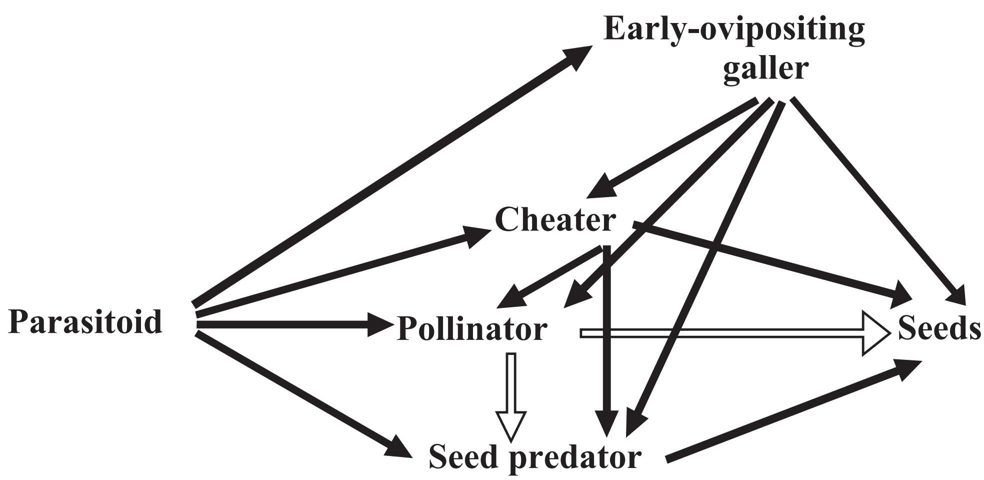


Fig. 1. Distribution of sample sites (a) and path analysis model used to test the host-parasitoid relationships for each parasitoid species (b). (a) Triangles and squares represent sites in the plant's native and introduced ranges, respectively. (b) Arrows represent the directions of effects, with black and open arrows indicating potential negative and positive effects respectively.

between galls and seeds for nutrition, while pollinator offspring abundance was expected to be positively linked to seed production.

We only included putative parasitoids appearing in more than 20 figs in either the native or introduced ranges of *F. microcarpa* into the path analysis. For each putative parasitoid species, only figs containing that species were used. Any other fig wasp species that emerged from <30% of these figs were excluded. We did not test for evidence of secondary parasitism in the model (parasitoids developing at the expense of other parasitoids) because two parasitoid species seldomly shared the same fig.

Co-association. Path analysis was unlikely to detect interactions involving less common fig wasp species. We therefore also examined the co-occurrence of parasitoids and their putative hosts within individual figs as a supplementary approach. In a fig occupied by only one species of phytophage and one species of parasitoid, it can be assumed that the phytophage is the likely host, thereby allowing rarer associations between parasitoids and phytophages to be identified. This nonetheless assumes that all individuals of alternative phytophagous hosts have not been killed by the parasitoid. To avoid such 'false positive' interactions, we only considered species-pairs that were recorded from at least three figs. Figs collected from both ranges were combined together because they were seldomly occupied only by a parasitoid-phytophage pair, and rare species that occurred in less than 10 figs were not considered.

Data analyses. All statistical analyses were carried out using R 3.3.3 setting a hierarchical random effect (figs nested in crops nested in study sites) (R Development Core Team 2017). Likelihood ratio (LR) tests and multiple tests with Bonferroni corrections were used to estimate the significance of fixed effects and pair-wise comparisons, respectively. Response variables were square root or natural logarithm transformed where necessary.

We compared the differences in species richness of fig wasps and fig wasp abundance per fig at different trophic levels and parasitoid prevalence between the native and introduced range of *F. microcarpa*, using Generalized Linear Mixed Models (GLMMs) in R package lme4 version 1.0-5 (Bates *et al*., 2013), assuming either Poisson or binomial distribution of residuals.

We tested food web structure of fig wasps in the two ranges of the plant based on the path analysis model (Fig. 1b) using Structural Equation Modeling (SEM) in R package piecewise SEM version 1.2.1 (Lefcheck, 2016), assuming Poisson distribution of residuals.

At both species and the generic levels, niche differentiation among phytophages and parasitoids with different hosts was examined by comparing the sizes and pedicel lengths of galls occupied by different fig wasp species/genera using Linear Mixed Models (LMMs) in R package nlme version 3.1 (Pinheiro *et al*., 2013). *Micranisa* and *Walkerella* (Otitesellinae) are closely related genera and were combined, and the seed predator *P. taiwanensis* was not included in the *Philotrypesis* spp. Data from both ranges were combined due to limited sample size of parasitoids in the plant's introduced range.

Results

Fig wasp community

We recorded the contents of 2681 figs from 192 crops, including 857 and 1824 figs in the native and introduced ranges of *F. microcarpa*, and a total of 99038 adult fig wasp offspring were present. We identified a total of 1 pollinating and 31 NPFW morpho-species with 14 and 18 species provisionally identified as phytophages and parasitoids respectively (Table S2). All morpho-species were detected in figs within the native range of *F. microcarpa* except three species (*Sycobia* sp., *Bruchophagus sensoriae* Chen and *Ormyrus* sp.). Although only eight parasitoid species were present in the plant's introduced range, most of the phytophagous species were recorded there. The only absences were *Walkerella nigrabdomina* Ma & Yang and *Walkerella* sp. (Table S2).

The mean species richness per fig in the plant's native range was significantly higher than that in the introduced range (Table 1). The mean phytophagous species richness was similar in both ranges, but a far higher parasitoid species richness was recorded in the plant's native range (Table 1; Fig. S1). In addition, parasitoids were absent in most figs in the plant's introduced range, while less than half of the figs did not contain parasitoids in the native range of *F. microcarpa*, indicating a significant difference in prevalence (Table 1; Fig. S1). There was no significant difference in both total fig wasp abundance and abundance of phytophages between the two ranges, whereas parasitoids in the plant's native range were much more abundant than those in its introduced range (Table 1).

Path analysis

In the plant's native range, four common Sycoryctinae putative parasitoids had specific negative correlations with the pollinating agaonids, and in addition *Philotrypesis okinavensis* Ishii and *Sycoscapter gajimaru* Ishii were also negatively associated with *Walkerella microcarpae* Bouček and *Eupristina* sp. respectively. *Philotrypesis emeryi* Grandi imposed the strongest negative effect on the pollinator based on path coefficients (Table S3; Fig. 2a). Another Sycoryctinae species, *Sycoryctes* sp., which has a very limited geographical distribution, only negatively correlated with the 'cheater' *Eupristina* sp. (Table S3; Fig. 2a). *Odontofroggatia* spp. were the specific hosts of *Sycophila* spp., and *Odontofroggatia galili* Wiebes and *Odontofroggatia corneri* Wiebes were negatively correlated with three *Sycophila* parasitoids (*Sycophila maculafacies* Chen, *Sycophila maculafacies* 'pale' and *Sycophila petiolata* Chen) (Table S3; Fig. 2a). We failed to detect any negative associations between putative parasitoids and seeds and between putative parasitoids and the seed predator, *P. taiwanensis*, which had a strong negative impact on seed production (Table S3; Fig. 2a).

Only four of the eight parasitoids analysed in the plant' native range were available for path analysis in the introduced range, and we failed to detect any variation in their host ranges (Table S3; Fig. 2b). Between the two Sycoryctinae species, *S. gajimaru* exhibited a stronger negative effect on the pollinator than *P. okinavensis* (Table S3; Fig. 2b). In addition, the parasitoid *Bruchophagus sensoriae* Chen, which was only recorded outside the plant's native range, was exclusively negatively associated with the epichrysomallid gall former *Meselatus bicolor* Chen (Table S3; Fig. 2b).

Evidence for both inter-specific competition and facilitation among putative phytophages were present, but these were not consistent throughout all analyses in both ranges (Table S3; Fig 2a, b).

Table 1. Comparisons of species richness of fig wasps and fig wasp abundance per fig (mean ± SE) at different trophic levels and parasitoid prevalence between the native and introduced ranges of *Ficus microcarpa* based on likelihood ratio (LR) tests using GLMMs assuming either Poisson or binomial distribution of residuals.

				Native versus Introduced range	
	Overall	Native range	Introduced range	d.f.	LR
Total species richness	1.96 ± 0.02	2.65 ± 0.05	1.64 ± 0.02	1	7.47**
Species richness of phytophages	1.55 ± 0.02	1.79 ± 0.03	1.44 ± 0.02	1	1.49^{NS}
Species richness of parasitoids	0.41 ± 0.01	0.86 ± 0.03	0.20 ± 0.01	1	9.68**
Total fig wasp abundance	36.94 ± 0.64	50.14 ± 1.27	30.74 ± 0.68	1	3.43^{NS}
Phytophage abundance	2.41 ± 0.11	4.79 ± 0.25	1.29 ± 0.09	1	1.91^{NS}
Parasitoid abundance	34.16 ± 0.65	45.18 ± 1.32	28.99 ± 0.68	1	9.41**
Parasitoid prevalence (%)	29.32	53.44	17.98	1	10.40**

NS: not significant.
$^{**}P < 0.01$.

Species associations

Using the figs that contained combinations of one parasitoid and one phytophage species, we identified a total of 15 parasitoid-phytophage associations including two extra trophic interactions, that is, *S. maculafacies* and *Odontofroggatia quinifuniculus* Feng & Huang, and *S. gajimaru* and *W. microcarpae* (Table S4).

Gall sizes

The volumes of 1261 galls occupied by 18 fig wasp species were obtained from 105 *F. microcarpa* figs (Tables S5 and S6). Significant variations in natal gall size were detected among the galls occupied by different genera of phytophages and parasitoids (Table S7). *M. bicolor* and *B. sensoriae* were reared from extremely large galls with volumes at least 2.5 times those containing any other species (Table S5; Fig. 3a). We detected no within-genus variation in gall size in any of the phytophages and parasitoids (Table S8). Support for our identified associations between parasitoids and their particular hosts were provided by a lack of any differences in the sizes of galls containing phytophages and their putative parasitoids (Tables S9 & S10; Fig. 3a).

Spatial stratification of fig wasps within figs

Pedicel lengths of 2203 flowers from 33 figs were measured. They included 544 seeds, 98 failed galls, 31 seeds occupied by *P. taiwanensis* and 1530 galled ovules containing 15 other fig wasp species (Tables S5 & S6). No within-genus variation in host gall pedicel length was detected in any of the phytophages and parasitoids (Table S8). Agaonids and their parasitoids (*Philotrypesis* spp., *Sycoryctes* spp. and *S. gajimaru*) and Otitesellinae spp. and their parasitoids (*Philotrypesis* spp.) emerged mainly from the more central galls with longer pedicels, while *Odontofroggatia* spp. and their parasitoids (*Sycophila* spp.) tended to occupy ovules nearer to the fig wall (Tables S5–S7; Fig. 3b). Similar pedicel lengths of natal galls were found in each parasitoid host pair (Tables S9 and S10; Fig. 3b).

Discussion

This study has revealed the food web of fig wasps associated with *F. microcarpa* in both its native and introduced ranges and tested the factors contributing to the formation of the parasitoid host ranges. Path analysis and species associations revealed the major trophic links within the fig wasp community, with most parasitoids being specific at host genus level. Our results also offered evidence for competitive and facilitative interactions among phytophages. Parasitoids associated specifically with the pollinator were present, with the pollinator aggregate (and 'cheater' agaonid) from *F. microcarpa* being the hosts of five sycoryctine species, as has been recorded for pollinators associated with fig trees native to Africa, Australasia and South America (Compton, 1993a; Segar & Cook, 2012; Segar *et al.*, 2013). The smaller range of the *Eupristina* sp. 'cheater' meant that fewer interactions with parasitoids were detected, but its suite of parasitoids was otherwise similar to that of the pollinator. In addition, as recorded by Compton (1993b) in Africa, epichrysomallines were the exclusive hosts of eurytomids. The apparent absence from the plant's native range of *B. sensoriae*, a specific parasitoid of *M. bicolor*, requires further investigation, but may reflect a species that is rare, but not absent, there. No parasitoids were detected in association with *P. taiwanensis*. It is an example of a major shift to phytophagy from parasitoid ancestors, and utilisation of this novel resource appears to have provided it with 'enemy-free space' within the figs (e.g. Rodriguez *et al.*, 2015).

All parasitoids that are common to both ranges of *F. microcarpa* displayed consistent host ranges, suggesting that factors independent of locally varying environments

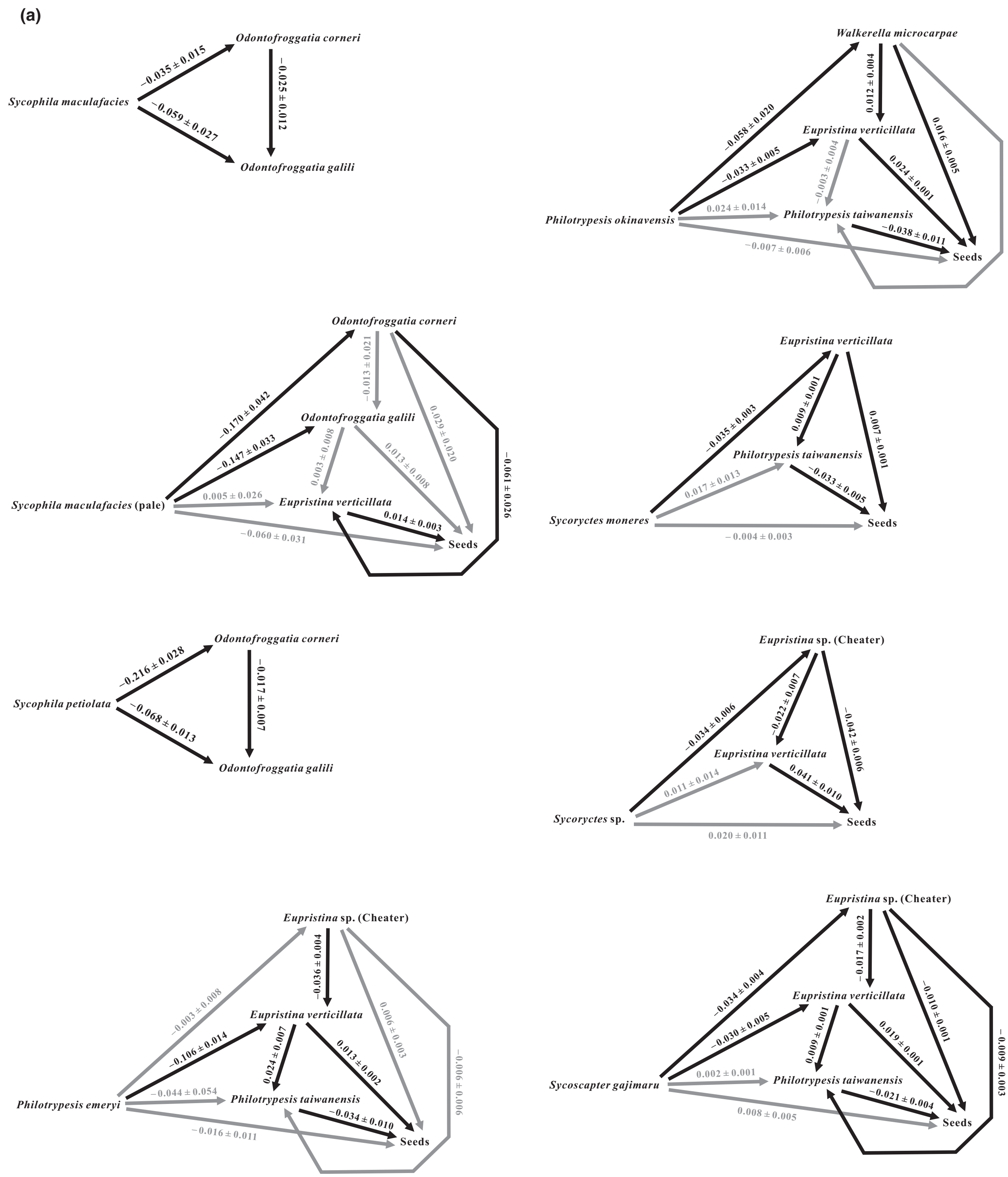


Fig. 2. Food web diagrams of the fig wasp community in the native (a) and introduced (b) range of *Ficus microcarpa* based on the results of SEM. Black and grey arrows represent significant and insignificant effects which were assumed as shown in Fig. 1, and path coefficients (mean ± SE) were provided for all effects.

play a role in determining these host-parasitoid relationships. Body size differences among fig wasps reflect the size of their galls, and size has been identified previously as a potential driver of galler-parasitoid specificity inside figs (Segar *et al.*, 2013). In this study, all genera of phytophages with different gall sizes supported distinct

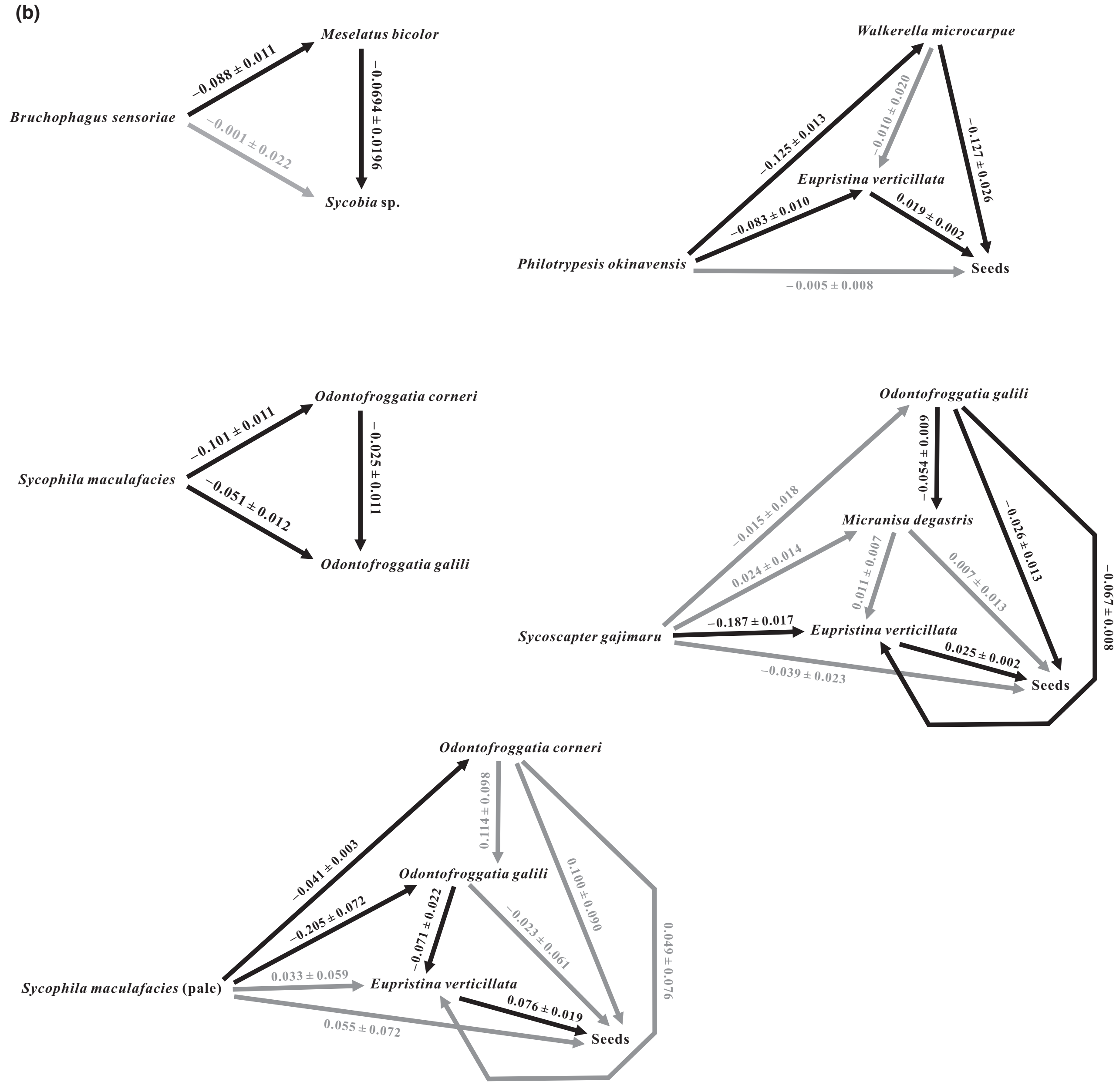


Fig. 2. Continued.

groups of parasitoids except for the Otitesellines. Otiteselline species produce galls that are slightly (though significantly) larger than those of agaonids, and were acting as hosts for some sycoryctines that usually develop inside the galls of agaonids. Given the high species richness of some Sycoryctinae genera (e.g. Zhou *et al.*, 2012) and their known trophic diversity (e.g. Wang *et al.*, 2014), it is likely that some species are moderately flexible in their host relationships.

Parasitoid host specificity to particular higher taxa has been described in previous fig wasp community studies (Dunn *et al.*, 2008), and suggests a co-evolutionary history between parasitoids and their hosts (Cook & Segar, 2010; Segar *et al.*, 2013). However, insofar as related species tend to generate similar-sized galls, it is hard to separate gall-size effects from phylogenetic history. Within groups with similar-sized galls, host specificity was not evident. For example, there was no evidence for particular *Sycophila* species being associated with individual *Odontofroggatia* species, whereas the related species (*M. bicolor*) that produce exceptionally large galls appears to evade *Sycophila* species. The widespread breakdown of host specificity at the host species level indicates a lack of niche differentiation within each gall-size group and suggests that gall size, rather than

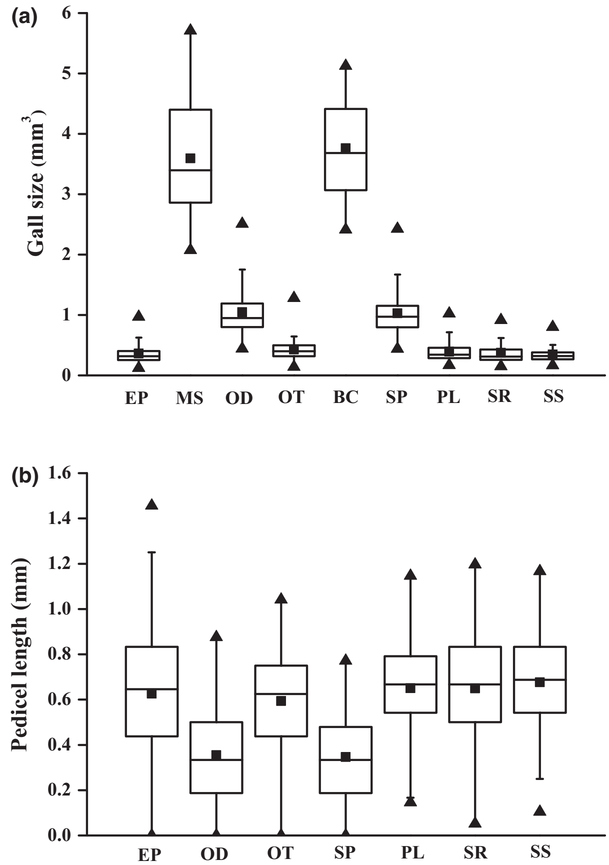


Fig. 3. Volumes (a) and pedicel lengths (b) of galls containing fig wasp species or genera. Line, box, whiskers, black squares and black triangles represent the median, the range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values of pedicel lengths in each utilisation type. EP *Eupristina* spp.; MS *Meselatus*; OD *Odontofroggatia* spp.; OT Otitesellinae spp.; BC *Bruchophagus*; SP *Sycophila* spp.; PL *Philotrypesis* spp. excluding *P. taiwanensis*; SR *Sycoryctes* spp.; SS *Sycoscapter*.

taxonomic affiliation *per se* is the main driver of host relationships in *F. microcarpa* figs. This pattern exists in some other fig wasp communities (Segar *et al*., 2013), but exceptions have also been reported, such as the *Apocrypta* parasitoid from *F. sur*, which utilises galls with varying sizes and displays a strikingly wide range of body sizes (Compton & Robertson, 1988).

Galls of different sizes are not distributed randomly within *F. microcarpa* figs. The concentration of larger galls towards the periphery and smaller galls towards the centre reflects variations in ovule selection by ovipositing females belonging to different species and possibly also differences in the extent to which they stimulate pedicel extension (Dunn *et al*., 2008; Yu & Compton, 2012). For parasitoids that oviposit at developing stages of figs, species associated with smaller galls therefore require longer ovipositors than those that utilise larger galls. Such spatial stratification of galls is therefore indicative of niche diversification of different fig wasps.

There is a rising awareness of the importance of mutualistic organisms in biological invasions (Richardson *et al*., 2000; Dickie *et al*., 2010). However, the host-specific species that can attack mutualists are still seldom considered for biological control. Parasitoids of pollinating agaonids can regulate pollinator populations (e.g. Suleman *et al*., 2013) and indirectly affect seed production by reducing the number of female pollinators entering figs, but in general they release greater impact on pollinator offspring density than on seed production (Dunn *et al*., 2008; Segar & Cook, 2012). All the four Sycoryctinae species that utilised *E. verticillata* showed the same host ranges in both geographical ranges of *F. microcarpa*, but *P. okinavensis* and *S. gajimaru* were less specific to the pollinator, and *P. emeryi* imposed a stronger impact on the pollinator than *Sycoryctes moneres* based on path coefficients and is a potential candidate for aiding biological control of the tree. In addition, *P. taiwanensis* has the potential to be utilised together with the pollinator's natural enemies because this seed predator can significantly reduce seed production and is independent of parasitoids. Although our results provided a species pool for the biological control of *F. microcarpa*, it is essential to carry out risk assessments for all potential biocontrol agents, which includes rigorous pre-introduction testing and the reconstruction of their phylogenies to evaluate their adaptations, effects and invasiveness in the sites where *F. microcarpa* is invasive.

In conclusion, we have constructed the food web of common fig wasps associated with a widespread invasive fig species. The host ranges of parasitoid fig wasps were consistent in both native and introduced ranges of the plant and were compartmented by both the size and the locations of host galls. Based on their host specificity and effects on pollinator abundance and seed production, some species exhibited the potential to act as useful biocontrol agents though further studies are needed to ensure their safety and effectiveness.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12282:

Table S1. Study sites and sampling dates.

Table S2. Summary of fig wasp taxa associated with *F. microcarpa* (with abbreviations).

Table S3. Food webs established in the native and introduced ranges of *F. microcarpa* based on the results of path analyses using SEM.

Table S4. Combinations of fig wasp species where at least three figs contained only one parasitoid and one phytophage.

Table S5. Gall sizes and pedicel lengths (mean ± SE) of occupied flowers.

Table S6. Gall sizes (mean ± SE, mm^3) and pedicel length (mm) of genera.

Table S7. LMMs assessing differences in gall volumes and pedicel lengths among phytophage and parasitoid genera.

Table S8. Comparisons within each genus of gall size and pedicel length using LMMs.

Table S9. Results of pair-wise comparisons using LMMs of gall size and pedicel length for galler and parasitoid genera.

Table S10. Pair-wise comparisons of flowers occupied by pairs of gallers and parasitoids in terms of gall sizes and pedicel lengths (LMMs).

Figure S1. Frequency distributions of species richness of fig wasps per fig at different trophic levels in the native and introduced ranges of *F. microcarpa* (a–f).

References

Bates, D., Maechler, M. & Bolker, B. (2013) lme4: Linear mixed-effects models using S4 classes. *R package version 0.999999-2*. <http://CRAN.R-project.org/package=lme4> 20th October 2013.

Berg, C.C. & Corner, E.J.H. (2005) *Moraceae-Ficus*, Flora Malesiana, Ser. I, 17/2, Nationaal Herbarium Nederland, Leiden, the Netherlands, pp. 289–301.

Caughlin, T., Wheeler, J.H., Jankowski, J. & Lichstein, J.W. (2012) Urbanized landscapes favored by fig-eating birds increase invasive but not native juvenile strangler fig abundance. *Ecology*, **93**, 1571–1580.

Chen, Y.-R., Chuang, W.-C. & Wu, W.-J. (1999) Chalcid wasps on *Ficus microcarpa* L. in Taiwan (Hymenoptera: Chalcidoidea). *Journal of Taiwan Museum*, **52**, 39–79.

Chen, Y., Compton, S.G., Liu, M. & Chen, X.-Y. (2012) Fig trees at the northern limit of their range the distributions of cryptic pollinators indicate multiple glacial refugia. *Molecular Ecology*, **21**, 1687–1701.

Chen, H.-H., Yang, D.-R., Gu, D., Compton, S.G. & Peng, Y.-Q. (2013) Secondary galling: a novel feeding strategy among 'non-pollinating' fig wasps from *Ficus curtipes*. *Ecological Entomology*, **38**, 381–389.

Compton, S.G. (1993a) One way to be a fig. *African Entomology*, **1**, 151–158.

Compton, S.G. (1993b) An association between epichrysomallines and eurytomids (Hymenoptera: Chalcidoidea) in southern African fig wasp communities. *African Entomology*, **1**, 123–125.

Compton, S.G., Rasplus, J.-Y. & Ware, A.B. (1994) African fig wasp parasitoid communities. *Parasitoid Community Ecology*, pp. 343–368. (ed. by B.A. Hawkins and W. Sheehan), Oxford University Press, Oxford.

Compton, S.G. & Robertson, H.G. (1988) Complex interactions between mutualisms: ants tending Homopterans protect fig seeds and pollinators. *Ecology*, **69**, 1302–1305.

Compton, S.G., van Noort, S., Mcleish, M., Deeble, M. & Stone, V. (2009) Sneaky African fig wasps that oviposit through holes drilled by other species. *African Nature History*, **5**, 9–15.

Compton, S.G., Ball, A.D., Collinson, M.E., Hayes, P., Rasnitsyn, A.P. & Ross, A.J. (2010) Ancient fig wasps indicate at least 34 million years of stasis in their mutualism with fig trees. *Biology Letters*, **6**, 838–842.

Cook, J.M. & Rasplus, J.-Y. (2003) Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology and Evolution*, **18**, 241–248.

Cook, J.M. & Segar, S.T. (2010) Speciation in fig wasps. *Ecological Entomology*, **35**, 54–66.

Cruaud, A., Ronsted, N., Chantarasuwan, B., Chou, L.-S., Clement, W. L., Couloux, A., Cousins, B., Genson, G., Harrison, R. D., Hanson, P. E., Hossaert-McKey, M., Jabbour-Zahab, R., Jousselin, E., Kerdelhué, C., Kjellberg, F., Lopez-Vaamonde, C., Peebles, J., Peng, Y.-Q., Pereira, R. A. S., Schramm, T., Ubaidillah, R., vanNoort, S., Weiblen, G. D., Yang, D.-R., Yodpinyanee, A., Libeskind-Hadas, R., Cook, J. M., Rasplus, J.-Y. & Savolainen, V. (2012) An extreme case of plant-insect codiversification: figs and fig-pollinating wasps. *Systematic Biology*, **61**, 1029–1047.

Dickie, I.A., Bolstridge, N., Cooper, J.A. & Peltzer, D.A. (2010) Co-invasion by *Pinus* and its mycorrhizal fungi. *New Phytologist*, **187**, 475–484.

Dunn, D.W., Segar, S.T., Ridley, J., Chan, R., Crozier, R.H., Yu, D.W. & Cook, J.M. (2008) A role for parasites in stabilising the fig-pollinator mutualism. *PLoS Biology*, **6**, e59.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R., Soulé, M.E., Virtanen, R. & Wardle, D.A. (2011) Trophic downgrading of planet earth. *Science*, **333**, 301–306.

Feng, G. & Huang, D.-W. (2010) Description of a new species of *Odontofroggatia* (Chalcidoidea, Epichrysomallinae) associated with *Ficus microcarpa* (Moraceae) with a key to species of the genus. *Zootaxa*, **2335**, 40–48.

Gurevitch, J., Fox, G.A., Wardle, G.M., Inderjit, Taub, D. (2011) Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecology Letters*, **14**, 407–418.

Herre, E.A., Jandér, K.C. & Machado, C.A. (2008) Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Annual Review of Ecology, Evolution and Systematics*, **39**, 439–458.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, **17**, 164–170.

Kerdelhué, C., Rossi, J.P. & Rasplus, J.-Y. (2000) Comparative community ecology studies on old world figs and fig wasps. *Ecology*, **81**, 2832–2849.

Krishnan, A., Ghara, M., Kasinathan, S., Pramanik, G.K., Revadi, S. & Borges, R.M. (2015) Plant reproductive traits mediate tritrophic feedback effects within an obligate brood-site pollination mutualism. *Oecologia*, **179**, 797–809.

Lefcheck, J.S. (2016) PIECEWISESEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, **7**, 573–579.

Li, Y.-W., Zhou, X., Feng, G., Hu, H.-Y., Niu, L., Hebert, P.D.N. & Huang, D.-W. (2010) COI and ITS2 sequences delimit species, reveal cryptic taxa and host specificity of fig-associated Sycophila (Hymenoptera, Eurytomidae). *Molecular Ecology Resources*, **10**, 31–40.

Liu, M., Compton, S.G., Peng, F.-E., Zhang, J. & Chen, X.-Y. (2015) Movements of genes between populations: are pollinators more effective at transferring their own or plant genetic markers? *Proceedings of the Royal Society of London Series B: Biological Sciences*, **282**, 20150290.

Pereira, R.A.S., de Teixeira, S. & Kjellberg, F. (2007) An inquiline fig wasp using seeds as a resource for small male production: a potential first step for the evolution of new feeding habits? *Biological Journal of the Linnean Society*, **92**, 9–17.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D.; the R Development Core Team. (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-110.

R Development Core Team. (2017) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN3-900051-07-0.

Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J. & Rejmánek, M. (2000) Plant invasions - the role of mutualisms. *Biological Review*, **75**, 65–93.

Rodriguez, B.F.P. Jr., Gonzales, R.S. & Rodriguez, L.J.V. (2015) Fig wasps of *Philippine Ficus microcarpa* L.: Diversity and trophic Structure in an urban setting. *Philippines Agricultural Scientist*, **98**, 15–22.

Segar, S.T. & Cook, J.M. (2012) The dominant exploiters of the fig/pollinator mutualism vary across continents, but their costs fall consistently on the male reproductive function of figs. *Ecological Entomology*, **37**, 342–349.

Segar, S.T., Pereira, R.A.S., Compton, S.G. & Cook, J.M. (2013) Convergent structure of multitrophic communities over three continents. *Ecology Letters*, **16**, 1436–1445.

Shanahan, M., So, S., Compton, S.G. & Corlett, R. (2001) Fig-eating by vertebrate frugivores: a global view. *Biological Reviews*, **76**, 529–572.

Suleman, N., Raja, S. & Compton, S.G. (2013) Parasitism of a pollinator fig wasp: mortalities are higher in figs with more pollinators, but are not related to local densities of figs. *Ecological Entomology*, **38**, 478–484.

Sun, X.-J., Xiao, J.-H., Cook, J.M., Feng, G. & Huang, D.-W. (2011) Comparisons of host mitochondrial, nuclear and endosymbiont bacterial genes reveal cryptic fig wasp species and the effects of *Wolbachia* on host mtDNA evolution and diversity. *BMC Evolutionary Biology*, **11**, 86–93.

Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature*, **445**, 202–205.

Wang, R., Matthews, A., Ratcliffe, J., Barwell, L., Peng, Y.-Q., Chou, L.-S., Yu, H., Yang, H.-W. & Compton, S.G. (2014) First record of an apparently rare fig wasp feeding strategy: obligate seed predation. *Ecological Entomology*, **39**, 492–500.

Wang, R., Aylwin, R., Barwell, L., Chen, X.-Y., Chen, Y., Chou, L.-S., Cobb, J., Collette, D., Craine, L., Giblin-Davis, R.M., Ghana, S., Harper, M., Harrison, R.D., Mcpherson, J.R., Peng, Y.-Q., Pereira, R.A.S., Reyes-Betancort, A., Rodriguez, L.J.V., Strange, E., van Noort, S., Yang, H.-W., Yu, H. & Compton, S.G. (2015a) The fig wasp followers and colonists of a widely introduced fig tree, *Ficus microcarpa*. *Insect Conservation and Diversity*, **8**, 322–336.

Wang, R., Aylwin, R., Cobb, J., Craine, L., Ghana, S., Reyes-Betancort, J.A., Quinnell, R.J. & Compton, S.G. (2015b) The impact of fig wasps (Chalcidoidea), new to the Mediterranean, on reproduction of an invasive fig tree *Ficus microcarpa* (Moraceae) and their potential for its biological control. *Biological Control*, **81**, 21–30.

Wang, R., Compton, S.G., Quinnell, R.J., Peng, Y.-Q., Barwell, L. & Chen, Y. (2015c) Insect responses to host plant provision beyond natural boundaries: latitudinal and altitudinal variation in a Chinese fig wasp community. *Ecology and Evolution*, **5**, 3642–3656.

Yu, H. & Compton, S.G. (2012) Moving your sons to safety: galls containing male fig wasps expand into the centre of figs, away from enemies. *PLoS ONE*, **7**, e30833.

Zhou, M.-J., Xiao, J.-H., Bian, S.-N., Li, Y.-W., Niu, L.-M., Hu, H.-Y., Wu, W.-S., Murphy, R.W. & Huang, D.-W. (2012) Molecular approaches identify known species, reveal cryptic species and verify host specificity of Chinese *Philotrypesis* (Hymenoptera: Pteromalidae). *Molecular Ecology Resources*, **12**, 598–606.

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